

Research Article

Association of Oxidative Stress Markers with Atherogenic Index of Plasma in Adult Sickle Cell Nephropathy

M. A. Emokpae\(^1,2\) and P. O. Uadia\(^3\)

\(^1\) Department of Chemical Pathology, Aminu Kano Teaching Hospital, Kano 700001, Nigeria  
\(^2\) Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City 300001, Nigeria  
\(^3\) Department of Biochemistry, University of Benin, Benin City 300001, Nigeria

Correspondence should be addressed to M. A. Emokpae, biodunemokpae@yahoo.com

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This paper evaluates the association of oxidative stress and atherogenic index of plasma in order to assess the cardiovascular risk in Sickle cell nephropathy especially as lipoprotein levels are lower in SCD than non-SCD patients. Antioxidant enzymes, malondialdehyde (MDA), urea, creatinine, and glomerular filtration rate were evaluated in 110 confirmed sickle cell disease patients: 65 males in steady state, aged 21.1 ± 6.0 years, 30 males with macroalbuminuria, aged 24.5 ± 7.0, years and 15 with chronic kidney disease (CKD), aged 31.8 ± 2.0 years. The mean activity levels of glutathione peroxidase (GPx), superoxide dismutase (Cu/ZnSOD), and catalase (CAT) were significantly lower (\(P<0.001\)) in SCD with macroalbuminuria and CKD while MDA was higher (\(P<0.001\)) in SCD with macroalbuminuria and CKD compared with controls. There was negative correlation between GPx (\(P<0.001\)), Cu/ZnSOD (\(P<0.02\)), and Atherogenic index of plasma in SCD with CKD, while MDA shows a positive correlation (\(P<0.001\)) with AIP in SCD with CKD. There was however no correlation between CAT and AIP. Decreased activity levels of antioxidant enzymes and low HDL-cholesterol concentration were confirmed in adult SCD with CKD in Nigerians. The increase oxidative stress and high atherogenic index in CKD may accelerate the process of cardiovascular complications in adult SCD patients. Atherogenic index of plasma was negatively correlated with antioxidant enzymes and positively with MDA.

1. Introduction

Sickle cell disease (SCD) is a haemoglobinopathy which is characterized by red blood cell rigidity, compromised perfusions, and tissue infarction [1]. The kidney of patients with SCD is affected both by haemodynamic changes of chronic and by consequences of vaso-occlusion within the renal medulla [2, 3]. Renal abnormalities in structure and function occur with increasing age of subject with SCD. The pathogenesis of SCD is due to polymerization of sickle red blood cell causing chronic haemolytic anaemia, vaso-occlusive crisis, and intravascular haemolysis. Sickle cell disease patients are susceptible to increased oxidative stress due to constant haemolysis of mutant red blood cells since haemoglobin acts as powerful catalyst for initiation of peroxidative reaction [4, 5]. Proteinuria is common in adult patients with SCD and we earlier reported a 28% prevalence of proteinuria in this group of patients in Nigeria [6]. Proteinuria is a progression factor in chronic kidney disease heralding a further deterioration in renal function [6]. Metabolic abnormalities, inflammation, and ischaemia may increase oxidative stress in sickle cell nephropathy (SCN). Increased oxidative stress in SCN due to increased pro-oxidative activity may lead to diminished antioxidant system [4, 7]. Increased oxidative stress is considered as an important pathogenic mechanism in the development of cardiovascular, cerebrovascular, and peripheral vascular complications [8, 9]. Autoperoxidation of polyunsaturated fatty acids (PUFAs) is initiated by free radicals, and the products which are oxidized in vivo to form malondialdehyde are capable of damaging membrane of biomolecules [9, 10]. Lipid abnormalities and increased oxidative stress in
The sera were stored at -80°C and analysis was done within two weeks of collection. The sera were allowed to clot and serum was collected aseptically and dispensed into a plain tube using combi-9 commercial dipstick, which was used as enrolment in the study. Random urine was obtained for findings were obtained using structured questionnaires. The study population was 110 confirmed SCD patients attending sickle cell disease clinic of Aminu Kano Teaching Hospital. They consisted of 65 males in steady state, aged 21.1 ± 6.0 years, 30 males with macroalbuminuria aged 24.5 ± 7.0 years, and 15 with chronic kidney disease (CKD), aged 31.8 ± 2.0 years. Demographic and clinical examination findings were obtained using structured questionnaires. The study protocol was approved by the institute’s ethical committee and the patients gave informed consent before enrolment in the study. Random urine was obtained for analysis using combi-9 commercial dipstick, which was used to test for biochemical urinalysis. Five milliliter of blood was collected aseptically and dispensed into a plain tube after 12-hour fast. The blood was allowed to clot and serum obtained after centrifugation at 3000 rpm for 10 minutes. The sera were stored at -20°C and analysis was done within two weeks of collection. Urea was determined using urease colorimetric technique, creatinine was assayed using sodium hydroxide-picric acid technique, and superoxide dismutase (Cu/ZnSOD), and glutathione peroxidase (GPx) were assayed using ELISA kits supplied by Northwest life science specialities, Vancouver, Canada. Catalase was estimated using kit by SIGMA (St. Louis, Missouri, USA) and malondialdehyde was determined using thiobarbituric acid reacting substance kit supplied by Northwest life science specialities. Total cholesterol and triglyceride (TG) were determined using enzyme-catalyzed colorimetric methods by Randox laboratories, UK. HDL cholesterol was assayed using the supernatant after precipitation with magnesium chloride-phosphotungstic acid solution, while LDL cholesterol was calculated using Friedewald formula [11]. Cardiovascular risk ratio was calculated using atherogenic index of plasma (AIP) [12], which was defined as log(TG/HDL-c) with TG and HDL-c expressed in molar concentration. Glomerular filtration rate was estimated using Cockcroft-Gault formula [13]. Chronic kidney disease was defined as estimated glomerular filtration rate (eGFR) of <60 mL/min and presence of macroalbuminuria. Macroalbuminuria was defined as presence in urine of albumin concentration of ≥300 mg/L. A two-sample t-test was used to determine the statistical significance of the means between the different groups. A P value of 0.05 or less was considered statistically significant. Pearson correlation coefficient was used to show the levels of association of antioxidant enzyme levels with AIP in SCD patient with CKD.

3. Results

The results are as indicated in Tables 1, 2, and 3. Table 1 shows biochemical and lipoprotein levels of SCD subjects in steady state used as controls, SCD with macroalbuminuria, and CKD. The mean serum urea and creatinine in SCD with macroalbuminuria and CKD were significantly higher (P < 0.001) than those of the SCD control subjects while the mean eGFR was significantly lower (P < 0.001) in SCD with macroalbuminuria and CKD compared with control subjects. The mean levels of triglyceride, total cholesterol, LDL cholesterol, and VLDL cholesterol were significantly higher (P < 0.001) in CKD compared with SCD controls while HDL cholesterol was lower (P < 0.001) in CKD compared with control subjects. The atherogenic index significantly increased in SCD patients with CKD compared with SCD with macroalbuminuria and control group.

Table 2 indicates changes in oxidative stress markers in the study group. The mean activity levels of GPx, Cu/ZnSOD, and CAT were significantly lower (P < 0.001) in SCD with macroalbuminuria and CKD while MDA was higher.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SCD controls</th>
<th>SCD macroalbuminuria</th>
<th>SCD CKD</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>65</td>
<td>30</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.1 ± 6.0</td>
<td>24.5 ± 7.0</td>
<td>31.8 ± 2.0*</td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>2.6 ± 0.9</td>
<td>8.3 ± 2.0*</td>
<td>14.2 ± 2.6*</td>
<td>1.7–8.3</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>57.3 ± 9.8</td>
<td>260 ± 25*</td>
<td>498 ± 75*</td>
<td>53–116</td>
</tr>
<tr>
<td>eGFR (mL/min)</td>
<td>101 ± 2.3</td>
<td>72 ± 5.0*</td>
<td>15.1 ± 2.0*</td>
<td>90–128</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.16 ± 0.4</td>
<td>1.20 ± 0.2</td>
<td>1.7 ± 0.25*</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.06 ± 0.5</td>
<td>3.45 ± 0.6*</td>
<td>3.9 ± 0.34*</td>
<td>3.1–6.2</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.72 ± 0.2</td>
<td>0.70 ± 0.3</td>
<td>0.06 ± 0.08*</td>
<td>0.8–1.9</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>1.90 ± 0.5</td>
<td>1.8 ± 0.32*</td>
<td>1.82 ± 0.06*</td>
<td>&lt;3.99</td>
</tr>
<tr>
<td>VLDL-cholesterol (mmol/L)</td>
<td>0.47 ± 0.06</td>
<td>0.52 ± 0.06*</td>
<td>0.74 ± 0.06*</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>AIP</td>
<td>0.22</td>
<td>0.23</td>
<td>0.45</td>
<td>&lt;1.4</td>
</tr>
<tr>
<td>TC: HDL</td>
<td>2.67</td>
<td>2.57</td>
<td>3.03</td>
<td>&lt;2.4</td>
</tr>
</tbody>
</table>

eGFR: estimated glomerular filtration rate; AIP: atherogenic index of plasma; *P < 0.001.

SCN may accelerate the process of atherosclerosis in patients with SCN. This study evaluates the association of oxidative stress and atherogenic index of plasma in order to assess the cardiovascular risk in SCN especially when lipoprotein levels are lower in SCD than non-SCD patients.

2. Materials and Methods

The study population was 110 confirmed SCD patients attending sickle cell disease clinic of Aminu Kano Teaching Hospital. They consisted of 65 males in steady state, aged 21.1 ± 6.0 years, 30 males with macroalbuminuria aged 24.5 ± 7.0 years, and 15 with chronic kidney disease (CKD), aged 31.8 ± 2.0 years. Demographic and clinical examination findings were obtained using structured questionnaires. The study protocol was approved by the institute’s ethical committee and the patients gave informed consent before enrolment in the study. Random urine was obtained for analysis using combi-9 commercial dipstick, which was used to test for biochemical urinalysis. Five milliliter of blood was collected aseptically and dispensed into a plain tube after 12-hour fast. The blood was allowed to clot and serum obtained after centrifugation at 3000 rpm for 10 minutes. The sera were stored at -20°C and analysis was done within two weeks of collection. Urea was determined using urease colorimetric technique, creatinine was assayed using sodium hydroxide-picric acid technique, and superoxide dismutase (Cu/ZnSOD), and glutathione peroxidase (GPx) were assayed using ELISA kits supplied by Northwest life science specialities, Vancouver, Canada. Catalase was estimated using kit by SIGMA (St. Louis, Missouri, USA) and malondialdehyde was determined using thiobarbituric acid reacting substance kit supplied by Northwest life science specialities. Total cholesterol and triglyceride (TG) were determined using enzyme-catalyzed colorimetric methods by Randox laboratories, UK. HDL cholesterol was assayed using the supernatant after precipitation with magnesium chloride-phosphotungstic acid solution, while LDL cholesterol was calculated using Friedewald formula [11]. Cardiovascular risk ratio was calculated using atherogenic index of plasma (AIP) [12], which was defined as log(TG/HDL-c) with TG and HDL-c expressed in molar concentration. Glomerular filtration rate was estimated using Cockcroft-Gault formular [13]. Chronic kidney disease was defined as estimated glomerular filtration rate (eGFR) of <60 mL/min and presence of macroalbuminuria. Macroalbuminuria was defined as presence in urine of albumin concentration of ≥300 mg/L. A two-sample t-test was used to determine the statistical significance of the means between the different groups. A P value of 0.05 or less was considered statistically significant. Pearson correlation coefficient was used to show the levels of association of antioxidant enzyme levels with AIP in SCD patient with CKD.
Table 2: Oxidative stress markers of sickle cell disease control, macroalbuminuria, and chronic kidney disease.

<table>
<thead>
<tr>
<th>Oxidative markers</th>
<th>SCD controls</th>
<th>SCD with macroalbuminuria</th>
<th>SCD with chronic kidney disease</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>65</td>
<td>30</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Glutathione peroxidase (mU/mL)</td>
<td>9.2 ± 0.9</td>
<td>5.54 ± 3.5*</td>
<td>3.01 ± 0.24*</td>
<td>9.2–19.6</td>
</tr>
<tr>
<td>Superoxide dismutase (ng/mL)</td>
<td>30.6 ± 5.1</td>
<td>21.3 ± 5.2*</td>
<td>18.5 ± 2.6*</td>
<td>22.5–103</td>
</tr>
<tr>
<td>Catalase (µmol/min/L)</td>
<td>154 ± 7.9</td>
<td>150 ± 2.58</td>
<td>147 ± 1.06*</td>
<td>156–182</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>2.5 ± 0.4</td>
<td>3.8 ± 1.7*</td>
<td>5.30 ± 0.3*</td>
<td>0.025–0.98</td>
</tr>
</tbody>
</table>

*P < 0.001.

Table 3: Correlation between antioxidant enzymes and atherogenic index of plasma in SCD with chronic kidney disease.

<table>
<thead>
<tr>
<th>Correlation between parameters</th>
<th>R value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx and AIP</td>
<td>−0.760</td>
<td>0.001</td>
</tr>
<tr>
<td>Cu/ZnSOD and AIP</td>
<td>−0.621</td>
<td>0.02</td>
</tr>
<tr>
<td>CAT and AIP</td>
<td>−0.416</td>
<td>NS</td>
</tr>
<tr>
<td>MDA and AIP</td>
<td>0.943</td>
<td>0.001</td>
</tr>
</tbody>
</table>

GPx: Glutathione peroxidase; Cu/ZnSOD: Superoxide Dismutase; CAT: Catalase and MDA: Malondialdehyde.

(P < 0.001) in SCD with macroalbuminuria and CKD compared with controls.

The observed increases (P < 0.001) in the levels of serum triglyceride, LDL, VLDL, and decrease (P < 0.001) of HDL-cholesterol levels in SCD patients with CKD are consistent with other studies elsewhere and in Nigeria [27–30]. The lipid profile of SCD patients is quite lower than that in subjects with normal haemoglobin and SCD patients in Nigeria have even lower levels than SCD patients in America and the Middle East [27–29]. Several reasons have been proposed as to why there are inconsistencies between lipid studies; these include differences in age, diet, weight, smoking, gender, small sample sizes, different ranges of disease severity, and treatment regimen [31–33]. Shores et al. [28] proposed the reasons why lipoprotein levels are lower in SCD than non-SCD even in the same environmental condition to be partly due to a decrease in red blood cell volume leading to a dilution effect on plasma constituents. It may also result from strong downregulation of the synthetic pathways or downregulation of the enzymes in lipid biosynthesis. Others suggested that it is the haemolytic stress in SCD patients that is associated with a significant reduction in plasma lipids in SCD patients [29, 34].

5. Conclusion

Decreased activity levels of antioxidant enzymes and low HDL-cholesterol concentration were confirmed in adult SCD with CKD in Nigerians. The increase oxidative stress has been reported to mediate most of the risk factors involved in kidney disease [19]. Free radical mediated injury is the primary event leading to renal injury and progressive renal insufficiency and may lead to increased levels of lipid peroxidation. This occurs because cell membrane contains large quantity of PUFAs. These PUFAs react with ROS to form peroxide derivatives [20, 21]. Oxidative damage may alter both structure and function of the glomerulus due to its effects on mesangial and endothelial cells. Overexpression of ROS by both enzymatic and nonenzymatic pathways (Fenton chemistry) promotes intravascular oxidant stress that can disrupt nitric oxide homeostasis and produce the highly oxidative peroxynitrite [22]. Previous reports have shown that renal disease in SCD has the capacity to diminish arginine bioavailability through the loss of de novo arginine synthesis from citrulline which occurs primarily in the kidneys. Renal insufficiency may impair the major route for endogenous arginine biosynthesis [23] since it plays some roles in the regulation of nitric oxide production [24]. Adults with SCD are arginine deficient even at steady state [25, 26].

The observed increases (P < 0.001) in SCD patients with CKD as compared to healthy controls could be due to the significant increases in the levels of plasma constituents involved in oxidative stress processes. The portion of study population included is a single centre study and therefore may not adequately reflect the general population. The results obtained need further investigation in a larger population.
and high atherogenic index in CKD may accelerate the process of cardiovascular complications in adult SCD patients. Atherogenic index of plasma was negatively correlated with antioxidant enzymes and positively with MDA.

References


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